REMARKS

The present amendment and reply is submitted pursuant to 37 C.F.R. § 1.111. Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

The Office Action Summary correctly indicates that claims 44-72 are pending in the application.

By the present amendment, claims 44, 46, 47, 50, 56, 58, 69, and 71 have been amended.

Claim 44 has been amended to better describe the claimed subject matter. Support for the amendments to claim 44 can be found throughout the specification, and at least at page 7, line 33 to page 8, line 1, Example 1, in the claims as originally filed and claim 44 as previously described.

Claim 46 has been amended to better describe the claimed subject matter. Support for the amendment to claim 46 can be found throughout the specification, and at least at page 8, lines 20-23, in the claims as originally filed and claims 44 and 46 as previously described.

Claim 47 has been amended to better describe the claimed subject matter. Support for the amendment to claim 47 can be found throughout the specification, in the claims as originally filed, and claims 44 and 47 as previously described.

Claim 50 has been amended to better describe the claimed subject matter. Support for the amendment to claim 50 can be found throughout the specification, for example at

page 12, line 9 to page 13, line 27, in the claims as originally filed, and claims 44 and 50 as previously described.

Claim 56 has been amended to better describe the claimed subject matter by correcting a grammatical error. Support for the amendments to claim 56 can be found throughout the specification, in the claims as originally filed and claim 56 as previously described.

Claim 58 has been amended to better describe the claimed subject matter. Support for the amendments to claim 58 can be found throughout the specification and in at least the claims as filed and claim 58 as previously described.

Claim 69 has been amended to better describe the claimed subject matter. Support for the amendments to claim 69 can be found throughout the specification and in at least the claims as filed and claim 69 as previously described.

Claim 71 has been amended to better describe the claimed subject matter. Support for the amendments to claim 71 can be found throughout the specification and in at least the claims as filed and claim 71 as previously described.

By the present amendment, new claims 73-82 are added for examination.

Support for new claim 73 can be found throughout the specification, for example at in Figure 1, at page 27, line 32 to page 28, line 4, in Example 1 and in the claims as originally filed.

Support for new claims 74-75 can be found throughout the specification, for example in Figure 1, at page 27, line 20 to page 28, line 4, in Example 1 and in the claims as originally filed.

Support for new claims 76-79 can be found throughout the specification, for example at page 21, lines 31-31, in the claims as originally filed and as previously described.

Support for new claims 74-75 can be found throughout the specification, for example at page 27, line 20 to page 28, line 4, in the claims as originally filed, and claim 50 as previously described.

Support for new claim 82 can be found throughout the specification, for example at page 21, lines 31-31, in the claims as originally filed and as previously described.

Formal Drawings are being submitted concurrently herewith.

No prohibited new matter is believed to have been introduced by way of the above Amendments. Applicants reserve the right to file a continuation or divisional application directed to any subject matter that may have been canceled by way of the present Amendments.

Priority

The Official Action asserts that Applicants have not complied with one or more conditions for receiving the benefit of an earlier filing date. Specifically, the Official Action asserts that a reference to the prior application is required in the first sentence of the specification.

Applicants respectfully note that the present application was filed under 35 U.S.C. § 371 as the national stage of an international application. As such, the present application is entitled to the benefit of the earlier filing date of application FR 97 09152 under 35 U.S.C.

§ 365(b) subject to the requirements of 35 U.S.C. § 119(a) and the PCT and the Regulations under the PCT, which does not require such a reference in the specification. It is believed that all conditions for receiving the benefit of the earlier filing date of application FR 97 09152 have been complied with. Accordingly, Applicants respectfully request that the Office acknowledge the same.

Rejections under 35 U.S.C. § 112, first paragraph

Written Description

Claims 44-72 stand newly rejected under 35 U.S.C. § 112, first paragraph, as allegedly encompassing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The rejection is respectfully traversed.

Specifically, the Official Action alleges, at page 4, that the specification fails to define the term "non-oncogenic variants", which is recited in claims 47 and 61, or how to make and test for the variants. However, Applicants submit that this aspect of the invention is fully described in at least the following parts of the specification: In the paragraph bridging pages 9 and 10, such variants are defined as being those mutated in the region involved in the process of cell transformation. In this paragraph, such variants are described with reference to the knowledge in the art. The specification also teaches that the transforming power of E7 has been correlated with its capacity to bind the product of the retinoblastoma (Rb) gene. See, for example at page 2, lines 19-26, and page 31, lines 1-12.

The specification teaches that the transforming power of E6 has been correlated with its capacity to complex the product of the p53 gene. See, for example, page 2, lines 26-30. The region involved in this interaction has been identified between residues 111 and 115 of the native protein. See, for example, page 32, lines 4-7. Furthermore, the examples of the specification illustrate representative species of such non-oncogenic variants of HPV-16 E6 and E7 polypeptides, having residues 111 to 115 and residues 21 to 26, respectively, deleted.

Furthermore, in the paragraph bridging pages 9-10, several publications that further describe non-oncogenic variants of the HPV E6 and E7 polypeptides are cited. Given the knowledge of the sequences of the E6 and E7 polypeptides of various HPV strains and the high levels of homology found between some strains, one of skill in the art would appreciate that the principles taught in the present specification can be applied to polypeptides derived from HPV E6 and E7 generally.

In view of the teaching of the specification, taken together with the knowledge of the sequences of the E6 and E7 polypeptides, the structural and functional basis for the cell transforming capacity of the native polypeptides, and the high levels of homology between strains of HPV, one of skill in the art would recognize that the Applicants were in possession of the claimed invention, including this aspect of the invention, at the time the application was filed.

At page 5, the Official Action alleges that the specification fails to adequately describe the genus of immunogenic polypeptides homologous to SEQ ID NO:1 or SEQ ID NO:2 as recited in claims 49 and 62. The specification defines homologous as referring to

a degree of identity greater than 70%, with higher percentages preferred, at page 12, lines 5-8. The homology described in the specification reflects the degree of homology that can be used in making and using the embodiment of the invention described in claims 48 and 62.

The Official Action refers to the Encyclopedia Britannica (Online Version) and to Bowie et al., Everett et al., Scott et al., Bork, Marcotte et al., and Rudinger to show that polypeptides are inherently complex molecules. However, none of these references relate to the degree of predictability that a polypeptide homologous to a thoroughly studied immunogenic 243 or 185 amino acid polypeptide will also be immunogenic. While the effect of substitutions in a peptide hormone may be unpredictable, as the Official Action asserts is taught by Rudinger, and there may be challenges in computationally predicting the substrate of a transporter protein as the Official Action asserts is taught by Everett et at., these references do not relate to the adequacy of the description of the presently claimed invention.

The genus of polypeptides with at least a 70% homology to SEQ ID NO:1 or SEQ ID NO:2 is a mathematically defined genus. Choosing or recognizing a nucleotide sequence that encodes a homologous polypeptide sequence is well within the ability of a skilled artisan. Moreover, the skilled artisan would recognize structural aspects that correspond to the functional features of the polypeptides described in these claims.

It should be noted that a number of type-specific and cross-reactive epitopes of the E6 and E7 regions of HPV were known at the time the application was filed. Exemplary publications include (see abstracts attached as an Appendix):

1. Ressing et al., "Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides." *J Immunol.* 1995 Jun 1;154(11):5934-43.

2. Kast et al., "Role of HLA-A motifs in identification of potential CTL epitopes in human papilliomavirus type 16 E6 and E7 proteins." *J Immunol.* 1994 Apr 15;152(8):3904-12.

3. Stacey et al., "Scanning the structure and antigenicity of HPV-16 E6 and E7 oncoproteins using antipeptide antibodies." *Oncogene.* 1994 Feb;9(2):635-45.

4. Nindl et al., "Antibodies against linear and conformational epitopes of the human papillomavirus (HPV) type 16 E6 and E7 oncoproteins in sera of cervical cancer patients." *Arch Virol.* 1994;137(3-4):341-53.

5. Muller et al., "Identification of seroreactive regions of the human papillomavirus type 16 protein E4, E6, E7 and L1." *J Gen Virol.* 1990 Nov;71 (Pt 11):2709-17.

6. Selvey et al. "Identification of B-epitopes in the human papillomavirus 18 E7 open reading frame protein." *J Immunol.* 1990 Nov 1;145(9):3105-10.

7. Dillner J. "Mapping of linear epitopes of human papillomavirus type 16: the E1, E2, E4, E5, E6 and E7 open reading frames." *Int J Cancer.* 1990 Oct 15;46(4):703-11.

8. Tindle RW, et al., "Identification of B epitopes in human papillomavirus type 16 E7 open reading frame protein." *J Gen Virol.* 1990 Jun;71 (Pt 6):1347-54.

SEQ ID NO:1 comprises a non-oncogenic variant of HPV-16 E6 polypeptide fused with the secretory and membrane anchoring signals of the measles F virus. Given the knowledge in the art, the skilled practitioner would recognize portions of SEQ ID NO:1 that provide the immunogenic functionality. Many epitopes are linear; i.e. such epitopes require only the specific linear sequence. One of skill in the art would also recognize the presence of secretory and membrane anchoring sequences in a polypeptide homologous to the exemplary sequences. Thus, the description of functional elements serves to convey the essential features of the homologous sequences to one of skill in the art.

A number of polypeptides, which are too many to list, homologous to SEQ ID NO:1 and fulfilling all of the functional requirements to be within the scope of the claimed invention can be derived from a straightforward application of the description of the specification taken in view of the knowledge in the art. For example, the specification teaches that various secretory and membrane anchoring signals may be used in the present invention and describes some preferred species. See, for example, page 8, lines 10-28. In view of the teaching of the specification, one of skill in the art would recognize that swapping the secretory and/or membrane anchoring signals in SEQ ID NO:1 for another secretory and/or membrane anchoring signal would result in an immunogenic polypeptide homologous to SEQ ID NO:1 and posessing the required functional properties within the genus described by claims 47 and 61. Functional homologues of SEQ ID NO:2, as recited

in these claims, can be derived in the same manner. Accordingly, this aspect of the invention is adequately described when the specification is taken in view of the knowledge in the art.

For at least the foregoing reasons, it is clear that both the "non-oncogenic variants" recited in claims 47 and 61, and the polypeptides homologous to SEQ ID NO:1 or SEQ ID NO:2 as recited in claims 49 and 62 are more than adequately described, such that one of skill in the art would recognize that the inventors were in possession of the claimed invention at the time the application was filed. Withdrawal of the rejection of claims 44-72 as allegedly lacking written description is respectfully requested.

Enablement

Claims 44-72 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled because the specification purportedly does not enable any person skilled in the art to practice the invention commensurate in scope with the claims. At page 9, the Official Action asserts that the specification does not reasonably provide for treatment of *any* type of cancer in a subject by administering the claimed composition to a subject. At page 11, the Official Action again asserts that the specification fails to show *non-specific* antitumor response. The rejection is respectfully traversed.

By the present amendment, claims 44, 58, 69, and 71 are amended to recite that the claimed antitumoral composition is "for the treatment of an HPV related cancerous or precancerous condition." Thus, neither these claims or the claims dependant therefrom can be interpreted to imply that the composition can be used to treat *any* cancer. The intended

scope of use for the claimed compositions and methods is the treatment of an HPV related cancerous or precancerous condition. Applicants submit that the invention is fully enabled for the purpose described in the claims.

The specification demonstrates the enablement of the invention in working examples using art accepted models. The examples demonstrate regression of papillomavirus-induced tumors following administration of a vector which directs the expression of cell-surface anchored E6 and/or E7 HPV polypeptide(s), alternatively in combination with an immunostimulant. Such tumor protective effect has been shown for three different tumor models (BMK-16 myc cells in Example 3, E7W1 cells in Example 6 and TC-1 cells in Examples 8 and 9). In all cases, recombinant vectors producing membrane associated papillomavirus early antigens are more efficient than recombinant vectors producing native forms of the same antigens. Moreover various routes of administration and dosages have been explored, all confirming the superiority of the cell-surface anchored antigens. In the TC1 tumor grafted mouse model, protection achieves in certain conditions 100% both in therapeutical (Example 9 of the present application) and prophylactical (Example 8) conditions.

The Federal Circuit has cautioned the Office not to "confuse[] the requirements under the law for obtaining a patent with the requirements for obtaining government approval to market a particular drug for human consumption." *In re Brana*, 34 USPQ2d 1437, 1442 (Fed. Cir. 1995). The Federal Circuit also stressed that precedential authority "has determined that proof of an alleged pharmaceutical property for a compound by statistically significant tests with standard experimental animals is sufficient to establish

utility." *Id.* Thus, it is sufficient to show that a therapeutic or prophylactic immune response may be achieved in an appropriate animal model. No references have been cited in the record which show that the model systems used are not art accepted models now as they were at the time of the application.

The examples of the specification demonstrate enablement commensurate with the scope of the invention as claimed. In a previous Official Action (Paper No. 13), the Office acknowledged that the specification is enabling for treatment of cancer or a tumor in a subject by subcutaneous, intraperitoneal, intramuscular, or scarification delivery of a vaccinia vector encoding the HPV E6 or E7 proteins. Paper No. 13 also acknowledged enablement for prevention of cancer or a tumor in a mouse model. Applicants again note that an embodiment of the present invention has begun phase II clinical trials having demonstrated safety, tolerability, and evidence of producing an immune response. (Etheridge, *BioWorld International*, January 2, 2002, American Health Consultants.) The product (recombinant MVA expressing membrane-associated E6 and E7 HPV polypeptides and IL-2) has undergone Phase I clinical trials both in the USA and in Europe on patients with various stages of cervical lesions.

At page 9, the Official Action also alleges a lack of enablement resulting from the alleged lack of description of non-oncogenic variants, as recited in claims 47 and 61, and immunogenic polypeptides homologous to SEQ ID NO:1 and SEQ ID NO:2, as recited in claims 49 and 62. For at least the reasons set forth above, those aspects of the invention according to claims 47, 49, 61, and 62 are more than adequately described in the specification. Thus, given the teaching of the specification, taken with the knowledge of

one of skill in the art at the time the applications was filed, one of skill in the art would be able to make and use the invention as claimed.

For at least the foregoing reasons, and those presented in Papers No. 15 and 17, Applicants submit that the invention embraced by the present is fully enabled, and respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

Claim Rejections under 35 USC § 112, second paragraph

Claims 44, 47, 50-52, 58 and 59 stand rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite.

Claim 47 is alleged to be indefinite because the recitation of " said E6 or E7 polypeptide of a papillomavirus" purportedly lacks antecedent basis. By the present amendment, claim 47 is amended to more clearly indicate the antecedent basis of the polypeptide by reciting "said polypeptide encoded by the E6 or E7 early region of a papillomavirus genome".

Claim 50 is also alleged to be vague because the claim recites "wherein said recombinant vector comprises, in addition, the sequences encoding at least one compound." Without acceding to the assertion that a nucleic acid sequence can encode only a protein or peptide, but simply in order to expedite allowance, claim 50 is amended to recite "polypeptide" in place of "compound."

Applicants submit that one of skill in the art will be aware of the metes and bounds of the presently claimed invention. Accordingly, withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

No rejections under 35 U.S.C. § 102 or 103

The previous rejections over the prior art having been rendered moot, the Official Action does not raise any new rejections over the prior art. Therefore, the present claims are understood to be free of the prior art.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Christopher L. North
Registration No. 50,433

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620
Date: February 12, 2002

Attachment to Amendment dated February 12, 2003

Marked-up Claims 44, 46, 47, 50, 56, 58, 69 and 71

44. (Twice Amended) An antitumoral composition for the treatment of an HPV related cancerous or precancerous condition comprising at least one recombinant vector comprising sequence encoding at least one immunogenic polypeptide, wherein said polypeptide is a polypeptide naturally having a nonmembrane location and which is modified by inserting a membrane anchoring sequence,and if the natural polypeptide lacks a secretory sequence, inserting a secretory sequence, so as to have a membrane location at the surface of the cells in which it is expressed, wherein said vector is a non-integrative vector and wherein said immunogenic polypeptide is derived from a polypeptide encoded by the E6 or E7 early region of a papillomavirus genome.

46. (Amended) The antitumoral composition according to claim 44, wherein said membrane anchoring sequence and/or said secretory sequence is selected from the group consisting of rabies glycoprotein, HIV virus env glycoprotein, and measles virus F protein.

47. (Amended) The antitumoral composition according to claim 44, wherein said immunogenic polypeptide is derived from a nononcogenic variant of said [E6 or E7 polypeptide of a papillomavirus] polypeptide encoded by the E6 or E7 early region of a papillomavirus genome.

Attachment to Amendment dated February 12, 2003

Marked-up Claims 44, 46, 47, 50, 56, 58, 69 and 71

50. (Amended) The antitumoral composition according to claim 44, wherein said recombinant vector comprises, in addition, the sequences encoding at least one [compound] polypeptide which enhances the antitumoral effect of said composition.

56. (Twice Amended) A recombinant vector comprising [the] sequences encoding one or more immunogenic polypeptide(s), wherein at least one of said polypeptides is a polypeptide as defined in claim 44.

58. (Amended) A method [for treatment of cancer or a tumor] for the treatment of an HPV related cancerous or precancerous condition in a subject comprising administering an effective amount of the antitumoral composition of claim 47 to said subject to treat said cancer or tumor in said subject.

69. (Twice Amended) A method [for the treatment of cancer or a tumor] for the treatment of an HPV related cancerous or precancerous condition in a subject comprising administering an effective amount of the antitumoral composition according to claim 62 to said subject to treat said cancer or tumor in said subject.

71. (Amended) A method [for the treatment of cancer or a tumor] for the treatment of an HPV related cancerous or precancerous condition in a subject comprising

Attachment to Amendment dated February 12, 2003

Marked-up Claims 44, 46, 47, 50, 56, 58, 69 and 71

administering an effective amount of the viral particle according to claim 57 to said subject to treat said cancer or tumor in said subject.

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

1: J Immunol 1995 Jun 1;154(11):5934-43

Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides.

Ressing ME, Sette A, Brandt RM, Ruppert J, Wentworth PA, Hartman M, Oseroff C, Grey HM, Melief CJ, Kast WM.

Department of Immunohematology and Blood Bank, University Hospital Leiden, The Netherlands.

Human papillomavirus type 16 (HPV16) is strongly associated with cervical carcinogenesis. The HPV16 E6 and E7 oncoproteins are constitutively expressed in the majority of cervical tumor cells and are, therefore, attractive targets for CTL-mediated immunotherapy. In mice, the outgrowth of a lethal dose of HPV16-induced tumor cells has been prevented by vaccination with a CTL epitope encoded by HPV16 E7, indicating the feasibility of peptide immunization to obtain antitumor CTL responses. In the present study, the immunogenicity of 9 HLA-A*0201-binding peptides encoded by HPV16 E6 and E7 was analyzed in vivo in HLA-A*0201Kb transgenic mice and in vitro in CTL cultures induced from PBMC of HLA-A*0201+ healthy donors. Four peptides with a good binding affinity were immunogenic in HLA-A*0201Kb transgenic mice, and three of them were also highly immunogenic in CTL induction experiments with PBMC of HLA-A*0201+ healthy donors. Human CTL clones specific for these three peptides were capable of lysing the HPV16 E7-containing HLA-A*0201+ cervical carcinoma cell line CaSki. These E7-derived peptides (11-20, YMLDLQPETT; 82-90, LLMGTLGIV; 86-93, TLGIVCPI), therefore, are likely to represent naturally processed human CTL epitopes of HPV16. Additionally, these three HPV16-encoded peptides have the highest affinity of binding to the HLA-A*0201 molecule. In this study, peptides with a lower binding affinity were less immunogenic. Therefore, our data illustrate that the HLA-binding affinity of a peptide has a major impact on its immunogenicity. In conclusion, we have identified immunogenic peptides encoded by HPV16 E6 and E7 that could be used in vaccines for the prevention and treatment of cervical carcinoma.

PMID: 7538538 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

2: J Immunol 1994 Apr 15;152(8):3904-12

Role of HLA-A motifs in identification of potential CTL epitopes in human papillomavirus type 16 E6 and E7 proteins.

Kast WM, Brandt RM, Sidney J, Drijfhout JW, Kubo RT, Grey HM, Melief CJ, Sette A.

Department of Immunohematology, University Hospital Leiden, The Netherlands.

We have measured the binding affinity for five HLA-A alleles: HLA-A1 (A*0101), A2.1 (A*0201), A3 (A*0301), A11 (A*1101), and A24 (A*2401); of a set of all possible nonamer peptides (n = 240) of human papillomavirus type 16 E6 and E7 proteins. High affinity binding peptides were identified for each of the alleles, thus allowing us to select several candidates for CTL-based vaccines. Moreover, this unbiased set of peptides allowed an evaluation of the predictive value of HLA motifs derived either from the analysis of sequencing of pools of naturally processed peptides or from the binding analysis of polyalanine nonameric peptides that differed in the amino acids (aa) present at the anchor positions. Whereas pool sequencing-derived motifs were present in only 27% of high affinity binders, the more expanded motif, based on analysis of different aa substitutions at the anchor positions, was present in 73% of high affinity binders. Furthermore, it was found that the presence of anchor residues in a peptide was in itself not sufficient to determine binding to MHC class I molecules, because the majority of motif-containing peptides failed to bind to the relevant MHC. Finally, specific HLA motifs were used to predict peptide binders of 8, 10, and 11 aa in length. Several high affinity binding peptides were identified for each of the various peptide lengths, indicating a significant size heterogeneity in peptides capable of high affinity binding to HLA-A molecules.

PMID: 7511661 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

3: Oncogene 1994 Feb;9(2):635-45

Erratum in:
Oncogene 1994 May;9(5):1515

Scanning the structure and antigenicity of HPV-16 E6 and E7 oncoproteins using antipeptide antibodies.

Stacey SN, Eklund C, Jordan D, Smith NK, Stern PL, Dillner J, Arrand JR.

Cancer Research Campaign Department of Molecular Biology, Paterson Institute for Cancer Research, Christie Hospital, Manchester, UK.

The structure and antigenicity of the HPV-16 E6 and E7 oncoproteins was studied using a set of antisera against overlapping synthetic peptides. We report that antigenic, mobile regions of the native proteins, as defined by reactivity with antipeptide antisera, occur at the N-termini of both E6 and E7 proteins, corresponding to regions of known or suspected protein-protein interactions. The putative zinc finger domains were consistently non-reactive, despite computer predictions of relatively high antigenicity, suggesting that the proposed zinc finger regions are held in stable secondary structures that the peptides were not able to mimic. In E6, the linker region between the two zinc fingers was antigenic, indicating that the two zinc finger structures might be able to articulate relative to one another by a flexible linker region. The highly antigenic N-terminal region of HPV-16 E7 was also found to be antigenic in E7 of both HPV-11 and HPV-18, indicating that the E7 proteins of different HPV types have similar antigenic structures. The identification of antigenic regions of the E6 and E7 proteins should be therefore be useful in the design of site-directed antibodies against E6 and E7 for numerous HPV types.

PMID: 7507231 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

4: Arch Virol 1994;137(3-4):341-53

Antibodies against linear and conformational epitopes of the human papillomavirus (HPV) type 16 E6 and E7 oncoproteins in sera of cervical cancer patients.

Nindl I, Benitez-Bribiesca L, Berumen J, Farmanara N, Fisher S, Gross G, Lopez-Carillo L, Muller M, Tommasino M, Vazquez-Curiel A, et al.

Deutsches Krebsforschungszentrum, Forschungsschwerpunkt Angewandte Tumorvirologie, Heidelberg, Federal Republic of Germany.

Sera obtained from 137 cervical cancer patients were analysed for the presence of antibodies to the human papillomavirus (HPV) type 16 proteins E6 and E7 by the aid of different assays, i.e. ELISA using as antigen either synthetic peptides or the complete E7 protein and radio-immunoprecipitation (RIPA) which uses the viral protein made by in vitro transcription/translation. In agreement with previous reports, reactivity to the E7 protein was found more frequently than to the E6 protein (31.4% vs. 16.8%) when the sera were assayed by peptide-based ELISA. In contrast, when RIPA was employed, reactivity to either protein was obtained at similar frequency (38.7% vs 46.7%). When the protein was denatured prior to immuno-precipitation the reactivity was lost in all sera tested for E6-specific antibodies but only in a few samples in the E7-RIPA. Therefore it was concluded that the increased sensitivity of the E6-RIPA as compared to the E6 peptide-ELISA is due to the detection of antibodies to conformational epitopes which are presented by the in vitro product but not by the synthetic peptides. Eighty-two sera from healthy donors were tested by HPV 16E6- and E7-RIPA and also by ELISA using the HPV 16E7 protein which was produced in the fission yeast Schizosaccharomyces pombe. One sample reacted each in the E6- and E7-RIPA indicating a high specificity of these assays. The E7 protein-ELISA proved to be less sensitive for the detection of antibodies in cervical cancer patients' sera (22.6% positive) as compared to peptide-based ELISA or RIPA.

PMID: 7524466 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

5: J Gen Virol 1990 Nov;71 (Pt 11):2709-17

Identification of seroreactive regions of the human papillomavirus type 16 protein E4, E6, E7 and L1.

Muller M, Gausepohl H, de Martynoff G, Frank R, Brasseur R, Gissmann L.

Deutsches Krebsforschungszentrum, Heidelberg, F.R.G.

Small fragments of the DNA of human papillomavirus type 16 (HPV-16) were randomly cloned into the bacteriophage fd which expresses the resulting peptides as part of its capsid. Antisera raised against different HPV-16 fusion proteins were used for screening of the phage clones and the reacting peptides were determined by sequencing the inserted HPV-16 DNA fragments of the positive recombinants. Seroreactive regions of the proteins derived from the E4, E6, E7 (two regions) and L1 (three regions) open reading frames could be found by this approach. Of these seven regions, four were defined by at least two overlapping inserts, thus limiting the domains to between 10 and 15 amino acids. In the case of the E4 open reading frame, the same region identified by immunoscreening was also found when synthetic overlapping octapeptides were tested by ELISA with the anti-E4 antiserum. Using an approach to predict 'receptor-like' regions within the respective proteins, five of the seven regions were also identified. From the data on these regions, synthetic peptides were produced and used for the detection of antibodies against HPV-16 proteins in human sera by ELISA.

PMID: 1701482 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

6: J Immunol 1990 Nov 1;145(9):3105-10

Identification of B-epitopes in the human papillomavirus 18 E7 open reading frame protein.

Selvey LA, Tindle RW, Geysen HM, Haller CJ, Smith JA, Frazer IH.

Department of Medicine, University of Queensland, Princess Alexandra Hospital, Woolloongabba, Australia.

A panel of murine mAb raised against a MS2 replicase/HPV 18 E7 fusion protein included 23 reactive by ELISA with HPV 18 E7 determinants. A total of 19 of the 23 recognized linear epitopes in the N-terminal region of the E7 molecule, while the other four were deduced by binding inhibition assays to recognize conformational determinants in this region. All tested antibodies precipitated a 14-kDa peptide doublet that corresponded with the predicted size of the E7 protein from HeLa cells, but not from HPV 16 E7 containing CaSki cells. HPV 18 E7 protein was detected by immunolabeling with electron microscopy in both the nucleus and the cytoplasm of HeLa cells with the greater proportion occurring in the cytoplasm. No antibody reacted specifically by indirect immunofluorescence with HeLa cells. Weak cross-reactivity of some mAb with the E6 MS2-replicase fusion protein of HPV 16 was detected by ELISA, but no protein of the appropriate size was immunoprecipitated from CaSki cells. It is concluded that the B cell epitopes on the HPV 18 E7 transforming protein are located in the N-terminal region of the molecule and that some are weakly cross-reactive with HPV 16 E6 protein. E7 protein is either present in HeLa cells at a concentration too low to be detected by indirect immunofluorescence, or the N-terminal epitopes are masked by protein conformation or interaction with cellular or other viral components.

PMID: 1698872 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

7: Int J Cancer 1990 Oct 15;46(4):703-11

Mapping of linear epitopes of human papillomavirus type 16: the E1, E2, E4, E5, E6 and E7 open reading frames.

Dillner J.

Department of Virology, Karolinska Institute, Stockholm, Sweden.

Certain types of human papillomavirus (HPV), especially HPV type 16, are associated with proliferative lesions of the cervix uteri that can progress to malignancy. In order to map the linear epitopes of the HPV-encoded proteins, we have synthesized the predicted amino acid sequences of the open reading frames (ORFs) in the early region of HPV 16, as a set of 94 synthetic 20-residue peptides overlapping each other with 5 amino acids. The peptides were tested for reactivity with IgA, IgG and IgM antibodies in the sera of 30 patients with HPV 16-carrying cervical neoplasia. The E1 ORF had only low immunoreactivity, but several relatively minor epitopes were identified in the carboxyterminal part. The E2 ORF was found to contain several epitopes that were highly immunoreactive with a majority (up to 87%) of the cervical cancer patients' sera. The E4 ORF had one major, regularly IgA- and IgG-reactive epitope, whereas the E5 and E6 ORFs had only a few minor epitopes. The E7 ORF had several epitopes that were highly immunoreactive, but only with a minority of patients' sera. The 10 most immunoreactive peptides were also analyzed for immunoreactivity with 60 control sera, of which 22 were derived from patients with parotid gland tumors and 38 were derived from healthy volunteers. Most of the peptides were also immunoreactive with the control sera. However, the IgA antibodies, and to some extent the IgG antibodies, were found at much lower levels among the controls.

PMID: 1698732 [PubMed - indexed for MEDLINE]

Appendix to Amendment dated February 12, 2003

MEDLINE Abstracts

8: J Gen Virol 1990 Jun;71 (Pt 6):1347-54

Identification of B epitopes in human papillomavirus type 16 E7 open reading frame protein.

Tindle RW, Smith JA, Geysen HM, Selvey LA, Frazer IH.

Department of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia.

Human papillomavirus (HPV) type 16 is implicated in the aetiology of anogenital dysplasia which may progress to malignancy. HPV-16 DNA is actively transcribed in cervical carcinomas, the most abundant transcripts being from the E6 and E7 early open reading frames. The E7 protein has been shown to have transforming activity in vitro. In this report we define four immunodominant B epitopes within the protein corresponding to the E7 gene, using a panel of murine monoclonal antibodies. Three epitopes are linear and lie within the N-terminal region of the molecule, and are unique to the HPV-16 E7 protein. One epitope is non-linear and presumed to be conformational. At least three of the four epitopes of the E7 protein are detectable by immunoprecipitation from an HPV-16-infected cervical carcinoma cell line. The demonstrated immunogenicity of the E7 protein allows us to deduce that this molecule may be a potential candidate for incorporation in a vaccine against cervical cancer.

PMID: 1693666 [PubMed - indexed for MEDLINE]